

Application No.: 10/034,621

3

Docket No.: 564462001613

Amendment to the Claims:

Please cancel claims 13 to 15, without prejudice.

Please amend the claims as follows:

This listing of claims will replace all prior versions, and listing, of claims in the application:

Listing of Claims:

Claim 1 (previously presented): An isolated or recombinant nucleic acid comprising a sequence having at least 70% sequence identity to SEQ ID NO:1 and encoding a polypeptide having polymerase activity.

Claim 2 (previously presented): The isolated or recombinant nucleic acid of claim 28, wherein the polymerase activity is retained at the temperature for four or more hours.

Claim 3 (previously presented): The isolated or recombinant nucleic acid of claim 1, comprising a sequence as set forth in SEQ ID NO:1, or, sequences fully complementary thereto.

Claim 4 (Currently amended): An isolated or recombinant nucleic acid encoding a polypeptide having polymerase activity comprising (a) a sequence that hybridizes to a nucleic acid encoding a polypeptide having polymerase activity and having a sequence as set forth in SEQ ID NO:1, under hybridization conditions comprising about 42°C in 50% formamide, 5X SSPE, 0.3% SDS, and 200 ng/ml sheared and denatured salmon sperm DNA, and a wash step comprising a wash at 30 minutes at room temperature in a solution comprising 150 mM NaCl, 20 mM Tris hydrochloride, pH 7.8, 1 mM Na₂EDTA, 0.5% SDS, followed by a 30 minute wash in fresh solution, or, (b) a sequence fully complementary to (a).

Claim 5 (Currently amended): An isolated or recombinant nucleic acid encoding a polypeptide having polymerase activity comprising (a) a sequence that hybridizes to a nucleic acid encoding a polypeptide having polymerase activity and having a sequence as set forth in SEQ ID NO:1, under hybridization conditions comprising about 35°C in 35% formamide, 5X SSPE, 0.3%

Application No.: 10/034,621

4

Docket No.: 564462001613

SDS, and 200 ng/ml sheared and denatured salmon sperm DNA, and a wash in a buffer comprising 0.1X SSC, 0.5% SDS for 15 to 30 minutes at between the hybridization temperature and 68°C, and a wash step comprising a wash at 30 minutes at room temperature in a solution comprising 150 mM NaCl, 20 mM Tris hydrochloride, pH 7.8, 1 mM Na₂EDTA, 0.5% SDS, followed by a 30 minute wash in fresh solution, or, (b) a sequence fully complementary to (a).

Claim 6 (previously presented): The isolated or recombinant nucleic acid of claim 4, wherein the hybridization conditions further comprise a wash for about 30 minutes at room temperature in a buffer comprising 150 mM NaCl₂, 20 mM Tris hydrochloride, pH 7.8, 1 mM Na₂EDTA, 0.5% SDS, followed by a 30 minute wash in fresh buffer at T_m-10°C.

Claim 7 (Currently amended): ~~[[An]]~~ The isolated or recombinant nucleic acid having at least 70% sequence identity to the nucleic acid of claim 1, wherein the sequence identity is [[as]] determined by analysis with a sequence comparison algorithm.

Claim 8 (Currently amended): An isolated or recombinant nucleic acid that encodes a polypeptide having polymerase activity having at least 80% sequence identity to the nucleic acid of claim 1 an isolated or recombinant nucleic acid as set forth in SEQ ID NO:1 or a sequence fully complementary thereto.

Claim 9 (Currently amended): An isolated or recombinant nucleic acid that encodes a polypeptide having polymerase activity having at least 90% sequence identity to the nucleic acid of claim 8 an isolated or recombinant nucleic acid as set forth in SEQ ID NO:1 or a sequence fully complementary thereto.

Claim 10 (Currently amended): An isolated or recombinant nucleic acid that encodes a polypeptide having polymerase activity having at least 95% sequence identity to the nucleic acid of claim 9 an isolated or recombinant nucleic acid as set forth in SEQ ID NO:1, or, a sequence fully complementary thereto.

Application No.: 10/034,621

5

Docket No.: 564462001613

Claim 11 (previously presented): The isolated or recombinant nucleic acid of claim 7, wherein the sequence comparison algorithm is FASTA version 3.0t78 with the default parameters.

Claim 12 (Currently amended): An isolated or recombinant nucleic acid that encodes a polypeptide having polymerase activity comprising (a) at least ~~[[20]]~~ 100 consecutive bases of a sequence as set forth in SEQ ID NO:1, (b) at least ~~[[20]]~~ 200 consecutive bases of a sequence having at least 70% identity to SEQ ID NO:1 and encoding a polypeptide having a polymerase activity, or (c) sequences fully complementary to (a) or (b).

Claims 13 to 15 (Canceled)

Claim 16 (Currently amended): An isolated or recombinant nucleic acid encoding ~~[[a)]]~~ a polypeptide having polymerase activity and a sequence as set forth in SEQ ID NO: 2, or ~~[[b)]]~~ enzymatically active fragments of ~~(a)~~ having polymerase activity.

Claim 17 (Currently amended): An isolated or recombinant nucleic acid encoding a polypeptide having polymerase activity and comprising at least ~~[[20]]~~ 30 consecutive amino acids of ~~[[a)]]~~ a polypeptide having a sequence as set forth in SEQ ID NO: 2, or ~~(b) enzymatically active fragments of (a).~~

Claims 18 to 27 (canceled)

Claim 28 (previously presented): The isolated or recombinant nucleic acid of claim 1, wherein the polypeptide has a polymerase activity at a temperature in a range from about 90°C to 113°C.

Application No.: 10/034,621

6

Docket No.: 564462001613

Claim 29 (previously presented): The isolated or recombinant nucleic acid of claim 1, wherein the polypeptide has a polymerase activity at a temperature up to 150°C.

Claim 30 (previously presented): The isolated or recombinant nucleic acid of claim 1, wherein the polymerase activity comprises a DNA polymerase activity.

Claim 31 (previously presented): The isolated or recombinant nucleic acid of claim 1, wherein the polymerase comprises a 3'-5' exonuclease activity.

Claim 32 (previously presented): The isolated or recombinant nucleic acid of claim 1, wherein the polymerase lacks a 3'-5' exonuclease activity.

Claim 33 (previously presented): The isolated or recombinant nucleic acid of claim 1, wherein the polypeptide has a polymerase activity in salinity conditions from 5 mM to 200 mM salt.

Claim 34 (withdrawn): A method for amplifying a nucleic acid comprising using a polymerase as set forth in claim 1.

Claim 35 (withdrawn): The method of claim 35, wherein the amplification reaction is a polymerase chain reaction (PCR).

Claim 36 (previously presented): The isolated or recombinant nucleic acid of claim 1, wherein the nucleic acid further comprises an expression vector.

Claim 37 (previously presented): The isolated or recombinant nucleic acid of claim 36, wherein the expression vector comprises a viral particle, a baculovirus, a phage, a plasmid, a cosmid, a fosmid, a bacterial artificial chromosome, a viral DNA or a P1-based artificial chromosome.

Application No.: 10/034,621

7

Docket No.: 564462001613

Claim 38 (withdrawn): A method for identifying functional polypeptide fragments or variants encoded by fragments of SEQ ID NO:1, and sequences as set forth in claim 1, that retain the polymerase function of the polypeptide of SEQ ID NO: 2, and sequences substantially identical thereto, said assay comprising:

utilizing a polypeptide encoded by a nucleic acid having at least 70% sequence identity to SEQ ID NO: 1, and sequences substantially identical thereto, or polypeptide fragment or variant encoded by SEQ ID NO: 1, to effect DNA polymerase activity in a PCR amplification at extreme high temperature for four or more hours and under conditions that allow said polypeptide or fragment or variant to function, and

detecting formation of an amplification product, wherein formation of the amplification product is indicative of a functional DNA polymerase polypeptide or fragment or variant.

Claim 39 (previously presented): A method for making a polypeptide comprising:

- (a) providing a nucleic acid having a sequence set forth in claim 1 or claim 12; and
- (b) expressing the sequence, thereby expressing the polypeptide.

Claim 40 (previously presented) The method of claim 39, wherein the nucleic acid further comprises an expression vector.

Claim 41 (previously presented) The method of claim 39, further comprising inserting the nucleic acid into a host cell and expressing the sequence in the host cell.

Claim 42 (previously presented) The method of claim 41, wherein the host cell is a prokaryotic or a eukaryotic cell.

Claim 43 (previously presented): The method of claim 41, wherein the host cell is a yeast cell, a bacterial cell, a mammalian cell, a fungal cell, an insect cell or a plant cell.

Application No.: 10/034,621

8

Docket No.: 564462001613

Claim 44 (withdrawn): A method for producing a biologically active polypeptide and screening the polypeptide for enhanced activity by:

(a) introducing at least a first polynucleotide and a second polynucleotide, the at least first polynucleotide and second polynucleotide sharing at least one region of partial sequence homology, into a suitable host cell, wherein the first or second polynucleotide comprises a sequence as set forth in claim 1 or claim 12;

(b) growing the host cell under conditions which promote sequence reorganization, resulting in a hybrid polynucleotide;

(c) expressing a hybrid polypeptide encoded by the hybrid polynucleotide of (b);
and

(d) screening the hybrid polypeptide of (c) for biological activity under conditions which promote identification of enhanced biological activity.

Claim 45 (new): An isolated or recombinant nucleic acid encoding a polypeptide having polymerase activity comprising (a) a sequence that hybridizes to a nucleic acid having a sequence as set forth in SEQ ID NO:1, across the entire length of SEQ ID NO:1, under hybridization conditions comprising about 42°C in 50% formamide, 5X SSPE, 0.3% SDS, and 200 ng/ml sheared and denatured salmon sperm DNA, and a wash step comprising a wash at 30 minutes at room temperature in a solution comprising 150 mM NaCl, 20 mM Tris hydrochloride, pH 7.8, 1 mM Na₂EDTA, 0.5% SDS, followed by a 30 minute wash in fresh solution, or, (b) a sequence fully complementary to (a).